

**File S1.** Detailed information on the data and findings used for structural elucidation of 2-(4-(2-(phosphonoxy)ethyl)piperazinyl)-ethanesulfonic acid (PEPES).

Detailed evaluation of coeluting signals revealed highly-correlated  $m/z$  corresponding to  $[M+Na]^+$  and  $[M+K]^+$  adducts, confirming a molecular mass of 318.0654 Da (ppm error  $\approx 10$ ). The compound also produced an in-source fragmentation at  $m/z$  221.0954 (ppm error  $\approx 10$ ), which could be attributed to a loss of water from the protonated adduct of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, a buffering agent added to the cell cultures (HEPES). The mass difference between the molecular masses of this unknown compound and HEPES corresponded to a difference of  $HPO_3$  ( $m/z$  difference of 79.9639, ppm error  $\approx 10$ ), leading to the candidate formula  $C_8H_{19}N_2O_7PS$ , notable isotopic distribution scores were calculated ( $[M+H]^+$  ion, first candidate, >99% score). Evaluating the structure of HEPES, the most logical candidate compatible with the in-source fragment was a phosphorylation of HEPES free -OH moiety, giving 2-(4-(2-(phosphonoxy)ethyl)piperazinyl)-ethanesulfonic acid (PEPES). To further validate this annotation, tandem mass-spectrometry by a complementary LC-QqQ/MS analysis of iRBC samples in negative ESI mode. Targeting of the precursor ion for the potential PEPES ( $m/z$  317,  $[M-H]^-$ ), MS/MS spectra showed (i) a water loss from deprotonated PEPES, (ii) a peak corresponding to HEPES ( $m/z$  237), (iii) a characteristic fragment representing a phosphate loss ( $m/z$  219), and fragments corresponding to phosphate and metaphosphate ions ( $m/z$  79 and  $m/z$  97, respectively). These results collectively confirm the identity of the compound as PEPES corroborating that HEPES is selectively phosphorylated in *B. divergens* RBC cultures (Figures 6c and 6d).